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Stress-induced release of prolactin: Blockade by dexamethasone and naloxone may indicate β-endorphin mediation

(corticotropin/prolactin/naloxone/opiate receptor/morphine)

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ABSTRACT **Basal levels of immunoreactive (ir) β-endorphin, corticotropin (ACTH), and prolactin (PRL) in plasma of male rats decrease after dexamethasone pretreatment (400 μg/kg at 24 hr and 200 μg/kg at 2 hr before). Inescapable electric footshocks increase ir-β-endorphin, ACTH, and PRL plasma levels, and this effect is blocked by dexamethasone pretreatment. Morphine (20 mg/kg) also increases ir-β-endorphin, ACTH, and PRL levels. Dexamethasone pretreatment blocks the morphine-induced release of ir-β-endorphin but does not prevent the morphine-induced release of PRL. Naloxone, the opiate antagonist, decreases basal plasma levels of PRL and partially blocks the stress-induced increase of PRL, but it has no effect on the basal or stress-induced release of ir-β-endorphin. These results are consistent with the proposal that β-endorphin may interact with an opiate receptor involved in the regulation of PRL secretion.**

Since the early studies by Selye (1), corticotropin (ACTH) has been recognized as the primary pituitary hormone secreted in response to acute stress in all species studied. Nicoll *et al.* (2) recognized that prolactin (PRL) was also released by stress. Recently, we reported that immunoreactive (ir) β-endorphin was secreted in response to stress (3, 4). Moreover, we showed that ir-β-endorphin and ACTH were secreted concomitantly in nearly equimolar amounts.

β-Lipotropin, the pituitary hormone of which β-endorphin is amino acid sequence 61–91, was reported earlier to be released by stress (5). Although one recent report claimed that apparent β-endorphin immunoreactivity in human blood was due only to the crossreactivity of the antiserum with β-lipotropin (6), more recent reports from different laboratories have shown that, in fact, a β-endorphin-like substance does exist in human and rat blood and that its levels are higher after stress (7–11). Nevertheless, the role of β-endorphin in peripheral blood is still under scrutiny.

In this study, we examined the possibility that β-endorphin released by stress into the blood could promote the secretion of another pituitary hormone, PRL. In fact, several recent reports have suggested that PRL regulation may involve an endogenous opiate receptor. Morphine and the endogeneous opioid peptides [Met^5]enkephalin, [Leu^5]enkephalin, and β-endorphin all are stimuli of PRL release; on the other hand, naloxone, a pure morphine antagonist, decreases basal PRL levels (see refs. 12 and 13 for review).

In order to test the possibility that β-endorphin released by stress promotes PRL secretion through an opiate receptor, we compared the secretion of PRL and of β-endorphin. Secretion was provoked by stress or morphine after animals were pretreated with naloxone or dexamethasone. The latter, a potent synthetic glucocorticoid, is known to suppress the morphine and stress-induced release of ACTH (14, 15).

MATERIALS AND METHODS

Design of Experiments and Statistical Evaluation. The effects of dexamethasone pretreatment on footshock-induced release of β-endorphin were studied by using a two-way repeated measures design. In nine rats, a catheter (Corning Silastic) was implanted in the right jugular vein, passed under the skin, and mounted to a Luer needle hub on the skull by stainless steel screws and dental cement. This method allowed blood sampling during experimental manipulations without undue stress to the rats. Patency of catheters was maintained by daily flushings with physiological saline containing heparin (200 international units/ml). Three to 4 days were allowed for postoperative recovery. Dexamethasone (Decadron, Merck) pretreatment (n = 5) consisted of two subcutaneous injections—400 μg/kg at 24 hr and 200 μg/kg at 2 hr before footshocks. Blood samples (0.4 ml) were taken immediately before and 5, 10, 15, or 30 min after the onset of footshocks. An equal volume of heparin in saline (200 international units/ml) was injected after removal of blood samples, to avoid hypovolemia. Saturated Na_2EDTA (50 μl) was added to each blood sample, which was then immediately centrifuged in the cold; plasmas were frozen until assay.

In the remaining experiments, one-way designs were used, and the results were evaluated by use of the Student t range statistic. Effects of morphine (morphine sulfate, Merck), dexamethasone pretreatment, or both treatments on PRL and ir-β-endorphin blood levels were assessed in four groups of six rats. They were pretreated subcutaneously in order to avoid hypovolemia. Saturated Na_2EDTA (50 μl) was added to each blood sample, which was then immediately centrifuged in the cold; plasmas were frozen until assay. Effects of stress, dexamethasone pretreatment, or both treatments on PRL and ir-β-endorphin blood levels were evaluated in four groups of five rats pretreated with dexamethasone or saline as above. At time 0, animals were subjected to inescapable electric footshocks; blood samples were taken after 15 min of stress. The effects of stress, naloxone (provided by Endo Laboratories, Garden City, NY), or both treatments on PRL and ir-β-endorphin blood levels were also examined. Four groups of five rats were injected subcutaneously with saline or naloxone (10 mg/kg). Five minutes later, the animals were subjected to inescapable electric footshocks or left in their home cage. Blood samples were taken by decapitation 20 min after injection.

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Abbreviations: ir, immunoreactive; ACTH, corticotropin; PRL, prolactin.

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Subjects for all the experiments were 200-g male Sprague–Dawley rats (Simonson Farms, Gilroy, CA).

Radioimmunoassays. β-Endorphin and ACTH radioimmunoassays were performed as described (4). The radioimmunoassay for β-endorphin crossreacts 100% with β-lipotropin or pro-opiocortin (M_r 31,000). Nevertheless, gel filtration sizing experiments by ourselves (4) and others (6, 13) have shown that β-endorphin in rat plasma accounted for > 50% of the ir-β-endorphin. PRL assays were performed as described (16).

Stress. In all experiments with footshocks, rats with previously implanted jugular cannulae were placed individually in a Perspex box after the basal blood sample was withdrawn. Electric shock was delivered through the cage floor grid (1 mA, 1-sec duration, random 12 shocks/min) for 15 or 30 min, during which blood samples were taken; blood volumes were restored with heparin in physiological saline.

RESULTS AND DISCUSSION

Effects on ACTH and β-Endorphin Release. We previously reported that ACTH and ir-β-endorphin were concomitantly released in equimolar amounts in response to stress and proposed that all experimental procedures that activate the adenohypophyseal axis with increased ACTH blood levels would simultaneously increase ir-β-endorphin levels. Similarly, any other procedures altering ACTH blood levels would also be expected to alter ir-β-endorphin blood levels (3, 4). The first experiments in the present study were designed to test this relationship. In agreement with our previously reported results (3, 4), inescapable footshocks produced a highly significant increase in plasma ir-β-endorphin with the peak effect occurring 15 min after the onset (Fig. 1).

This form of stress also increases ACTH concomitantly (4). Pretreatment with dexamethasone blocked the stress-induced release of ACTH (14) and of ir-β-endorphin (Fig. 1). Morphine, which is known to be a strong stimulus for ACTH secretion (12, 15), also significantly increased plasma ir-β-endorphin in our experiments (Fig. 2). Morphine-induced release of ACTH, as reflected by corticosterone secretion, is blocked by dexamethasone pretreatment (15). Similarly, we found that dexamethasone also blocked morphine-induced release of ir-β-endorphin as well as ACTH release (morphine plus saline, ACTH = 1.28 ng/ml; dexamethasone + morphine, ACTH = 0.37 ng/ml). Pretreatment with dexamethasone decreased the basal levels of ACTH (saline controls, ACTH = 0.98 ng/ml; dexamethasone only, ACTH = 0.30 ng/ml) (see also ref. 4). Basal levels of ir-β-endorphin were also decreased by dexamethasone treatment (Figs. 2 and 3).

The observation that both stress and acute morphine treatment cause release of pituitary ACTH and ir-β-endorphin could

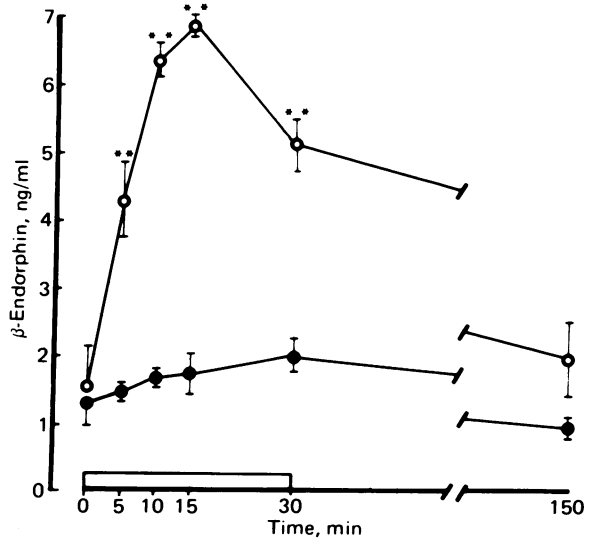

FIG. 1. Effect of stress on ir-β-endorphin blood levels after dexamethasone. Rats with indwelling catheters were used. Control rats (○; n = 4) and dexamethasone-pretreated rats (400 μg/kg 24 hr and 200 μg/kg 2 hr before) (●; n = 5) were subjected to inescapable electric footshocks for 30 min (0–30 min). Dexamethasone pretreatment completely blocked the footshock-induced increase of ir-β-endorphin. Data are shown as mean ± SEM. ** $P < 0.01$ for comparison to dexamethasone-pretreated rats.

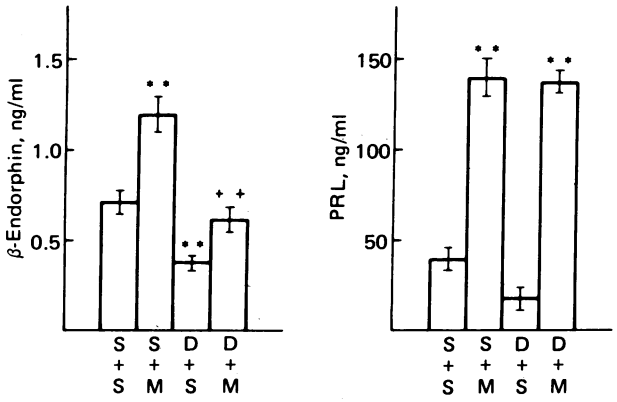

FIG. 2. Effects of morphine, dexamethasone pretreatment, and both treatments on PRL (*Right*) and ir-β-endorphin (*Left*) blood levels. Four groups of six rats were used and pretreated subcutaneously with dexamethasone (P) or saline (S). At time 0, animals were injected subcutaneously with either morphine sulfate (M) (20 mg/kg) or saline. Blood samples were taken by decapitation 15 min after this last injection. **, $P < 0.01$ for comparison with saline-treated control animals (S + S); ++, $P < 0.01$ for comparison with morphine-injected control animals (S + M).

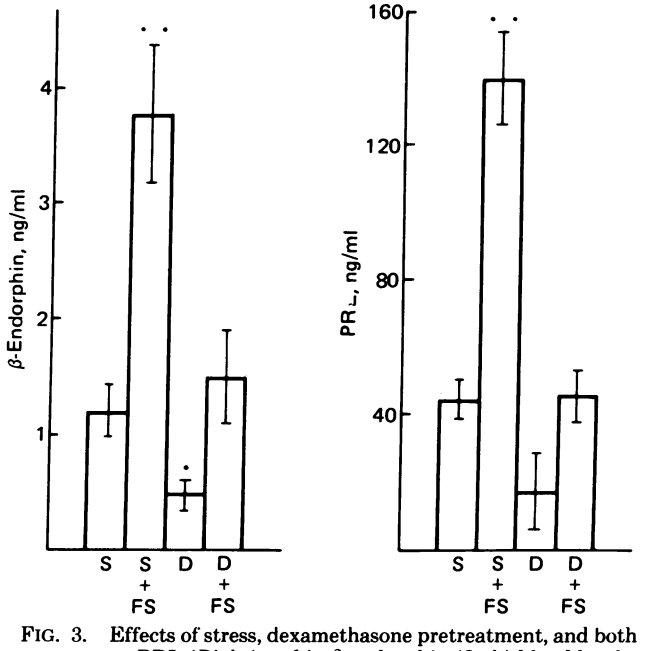

FIG. 3. Effects of stress, dexamethasone pretreatment, and both treatments on PRL (*Right*) and ir-β-endorphin (*Left*) blood levels. Four groups of five rats each were pretreated with dexamethasone (D) or saline (S) as in Fig. 2. At time 0, animals were subjected to inescapable electric footshocks (FS) as in Fig. 1. Blood samples were taken after 15 min of stress. * and **, $P < 0.05$ and $P < 0.01$, respectively, for comparison with saline-treated control (S); ++, $P < 0.01$ for comparison with saline-injected, footshocked animals (S + FS).

indicate that both perturbations act through a common molecular or cellular process. Indeed, Gibson *et al.* (17) suggested that the stress-induced release of ACTH and β-endorphin was mediated through an opiate receptor. However, as shown in Fig. 4, the opiate antagonist naloxone did not antagonize the stress-induced release of ir-β-endorphin even though this drug did antagonize morphine-induced release (not shown). Therefore, the stress-induced release of ir-β-endorphin cannot be mediated by conventional opiate receptors. Conversely, our results suggest that morphine itself may at least be a potent stressor whose naloxone-sensitive effects can induce a classical pituitary stress response, at least as such a response is defined by ACTH release (1).

Effects on PRL Release. The mechanisms regulating PRL secretion have also been proposed to include an opiate receptor (18); morphine and endogenous opioid peptides are potent stimuli of PRL release, whereas naloxone decreases basal PRL levels and also attenuates the stress-induced release of PRL (11, 12, 18, 19). Our second series of experiments evaluated this possibility with reference to experimental manipulations previously found to influence plasma levels of either ir-β-endorphin or PRL. Dexamethasone pretreatment decreases basal PRL levels markedly and blocks or attenuates the stress-induced release of PRL (refs. 20–25; see also Figs. 2 and 3).

Morphine also strongly stimulates PRL release (11, 12, 16), as might be expected from its presumed general stressor effects. However, in contrast with our finding that dexamethasone pretreatment suppresses morphine-induced release of ir-β-endorphin, we find now that dexamethasone pretreatment has no effect on the morphine-induced release of PRL (Fig. 2). This observation raises the possibility that opiate receptors may regulate PRL release at sites on lactotrophs not under corticosteroid regulation. Naloxone, the opiate antagonist, significantly decreased basal levels of prolactin ($P < 0.05$) and also significantly depressed its stress-induced release (refs. 11, 18, 19, and 26; also see Fig. 4). At some variance with the reports by Shaar *et al.* (26) and Van Vugt *et al.* (19), we found that naloxone decreased basal and stress-induced PRL levels at 10 mg/kg (Fig. 4) but not at 0.2 mg/kg (not shown). However, although these doses of naloxone had no significant effect on the basal levels of ir-β-endorphin, they did potentiate the stress-induced release of β-endorphin (Fig. 4). This finding may indicate that naloxone may potentiate the painful effects of footshocks through blockade of an endogenous opioid system involved with nociception. In contrast, these same doses of naloxone completely abolished the morphine-induced release of both ir-β-endorphin and PRL (not shown); indicating that these naloxone doses were adequate to antagonize morphine effects fully.

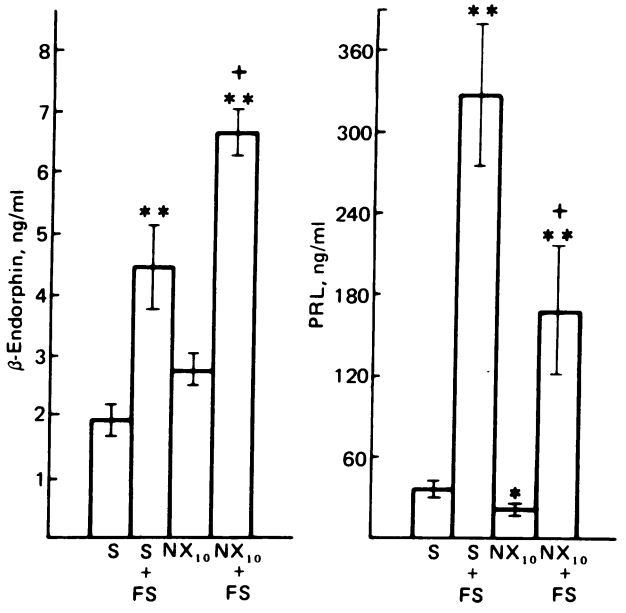

FIG. 4. Effects of stress, naloxone, or both treatments on PRL and ir-β-endorphin blood levels. Four groups of five rats each were injected subcutaneously with saline (S) or naloxone (NX_{10}, 10 mg/kg). Five minutes later, the animals were subjected to inescapable electric footshocks (FS) or left in their home cage. Blood samples were taken by decapitation 20 min after injection. * and **, $P < 0.05$ and $P < 0.01$ for comparison with saline-treated control (S); +, $P < 0.05$ for comparison with saline-injected footshocked animals (S + FS).

CONCLUSIONS

In this study, we assayed ir-β-endorphin and PRL simultaneously and observed that, under certain experimental conditions, the blood levels of these two pituitary hormones varied synchronously: (*i*) stress from footshocks or morphine increased blood levels of both ir-β-endorphin and PRL; (*ii*) dexamethasone pretreatment decreased basal levels of both ir-β-endorphin and PRL; and (*iii*) dexamethasone pretreatment abolished the PRL and ir-β-endorphin response to stress. Harms *et al.* (21) observed synchronous changes in ACTH and PRL blood levels in response to stress, adrenalectomy, and dexamethasone and proposed that a common mechanism may be involved in the pituitary release of ACTH and PRL.

We also observed that naloxone, like dexamethasone, substantially decreases basal PRL levels and abolishes the stress-induced release of PRL. However, dexamethasone usually has no direct action on normal pituitary lactotrophs *in vitro* (12, 13), even after treatments for 3 days. Similarly, neither the thyrotropin-releasing factor-induced release of PRL *in vitro* (27) nor the morphine-induced release of PRL (present results) are affected by dexamethasone pretreatment. Thus, stress, opiates, and naloxone each affect ACTH and ir-β-endorphin release identically under certain conditions *in vivo*. However, because PRL release can be modified by opiates in the presence of dexamethasone and because lactotrophs and corticotrophs are separate, distinct target cells, an alternative explanation to the common release mechanisms of ACTH and ir-β-endorphin must be sought for the associated release of PRL.

We now propose that stimuli that cause ir-β-endorphin to be released with ACTH could thereby also release PRL, with the β-endorphin acting as a natural agonist in the regulation of PRL secretion. Thus, when dexamethasone suppresses basal levels of β-endorphin, the putative endorphin receptor on lactotrophs will be relatively unoccupied and basal PRL secretion will decrease. Our other data further support this proposal in that morphine can induce secretion of PRL even in dexamethasone-pretreated rats although β-endorphin secretion does not increase. Furthermore, in the stress response we observed that naloxone significantly antagonized the release of PRL by stress. However, similar to findings of Van Vugt *et al.* (19), we found that naloxone did not completely antagonize the stress-induced release of PRL. Indeed, some effects of β-endorphin are not readily antagonized by naloxone (see refs. 28–30). The exact site of PRL secretion regulated by an opiate or endorphin is still unclear; because direct effects on pituitary PRL cells (16) seem unlikely, more likely sites could be the median eminence or the central nervous system (12, 16, 26).

The simultaneous induction of secretion of pituitary hormones by common stimuli may thus be seen to result from two different mechanisms: (*i*) pituitary hormones cosynthesized in a common precursor are secreted concomitantly from the same cells, as ACTH and β-endorphin/β-lipotropin (31, 32); and (*ii*)

one secreted hormone triggers the secretion of another, perhaps by a local endocrine mechanism, as β-endorphin and PRL.

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